HDAC inhibition releases the breaks on BTK targeting microRNA

Matthew D. Blunt and Andrew J. Steele, University of Southampton

In this issue of Blood*, Bottoni et al demonstrate that HDAC inhibition promotes the upregulation of microRNA which target BTK, subsequently suppressing pro-survival signaling in chronic lymphocytic leukemia (CLL) samples, and highlight a rationale for HDAC inhibitors in combination with ibrutinib for the treatment of patients.*[1]

B cell receptor (BCR) signaling is instrumental in the biology of CLL and kinase inhibitors (KI) such as ibrutinib and idelalisib, which target BTK and PI3Kδ respectively, are revolutionizing treatment of this disease. However, these agents are not curative, are taken indefinitely, and resistance and intolerance to these agents is already emerging. Ibrutinib discontinuation is associated with extremely poor survival and represents an important unmet clinical need. The primary resistance mechanism for ibrutinib is through mutations in BTK (C481S) or PLCγ2 (R665W and L845F),[2] however microenvironmental signals may also influence sensitivity to this drug.[3]

MicroRNA (miRNA, miR) are small double-stranded RNA molecules of ~19-23 nucleotides, identified as a mechanism cells use to regulate post-transcriptional expression of various genes and subsequently protein production. They do this by reducing mRNA stability, blocking mRNA translation or promoting mRNA degradation[4] and numerous publications have described important roles for miRNA in CLL pathogenesis and BCR-signalling.[5,6] Histone deacetylases (HDACs) regulate deacetylation and demethylation of lysine residues on histones resulting in the silencing of various genes. In CLL, HDACs inhibit expression of miRNA, particularly miR15a, miR16 and miR29b, which are associated with the suppression of Bcl-2 and Mcl-1.[7] CLL samples treated *in vitro* with HDAC inhibitors (HDACi) upregulated these miRNA and consequently suppressed Mcl-1 and Bcl-2 protein expression.[7]

In this issue of *Blood,* Bottoni et al hypothesized that treatment of CLL cells with HDACi may also regulate miRNA that target BTK. Indeed, they demonstrated that BTK protein expression was targeted and reduced most strongly by miR-210 and miR-425 in CLL, and that miR-210 and miR-425 were expressed at lower levels in primary CLL samples compared to normal B cells.[1] They suggested this was caused by silencing of BTK-targeting miRNAs by the HDAC repressor complex, indicating a novel outcome of epigenetic silencing, as well as providing a potential explanation for the overexpression of BTK in CLL. Furthermore, pharmacological or siRNA mediated targeting of HDAC1 activity increased the expression of BTK-targeting miRNAs and consequently reduced BTK expression and downstream signaling. Importantly these reductions were evident in BTK(C481S) mutated samples, indicating this approach may hold potential for the treatment of patients who become resistant to ibrutinib. Indeed the authors demonstrated the therapeutic potential of combining ibrutinib and HDACi in vitro on primary CLL cells and in vivo using the Eμ-TCL-1 mouse model, whereby responses were superior to either agent alone.[1] Importantly, computational biology predicts that pre-existing drug-resistant clones may exist in patients prior to ibrutinib treatment.[8] This indicates that the combination of HDACi with ibrutinib at least at therapy initiation may prevent/inhibit expansion of resistant clones during ibrutinib therapy, perhaps resulting in longer-term progression free survival. However, there are some potential concerns associated with targeting HDAC in CLL. Firstly, HDAC inhibitors have known toxicity in CLL patients[9] which may limit the usefulness of this approach. In particular, one side effect from treatment with HDACi is thrombocytopenia, therefore how this drug combination will affect bleeding-related events,

particularly following long-term usage and in combination with ibrutinib, still requires investigation. Indeed, Mato et al suggested an alternative KI may prove effective after resistance has emerged, although in that study ORR to a subsequent KI of 50% and a median PFS of 11.9 months suggested alternative salvage therapies require investigation.[10] In addition to the BTK(C481S) mutation, progressive disease in CLL whilst on ibrutinib can alternatively be attributed to PLCγ2 mutations (R665W and L845F) which promote BCR-downstream signaling independently of BTK. But how HDACi treatment will effect signaling/resistance associated with PLCγ2 mutations and whether this will select for PLCγ2 mutations in patients whilst on treatment, still remains to be evaluated.

In conclusion, this pioneering study identifies that epigenetic silencing of BTK-targeting miRNAs may contribute to BTK overexpression in CLL and demonstrates the possibility of using HDACi to remove/reduce the emergence of ibrutinib resistant clones. This study highlights the need to initiate clinical trials to assess this combination, given the urgent and unmet clinical need of kinase resistant CLL patients at present.

Figure 1 legend

Pharmacological inhibition of HDAC relieves epigenetic suppression of BTK targeting microRNA. A) HDAC repressor complex inhibits the function of miR-210 and miR-425, causing an upregulation of BTK. BCR ligation induces activation of BTK, promoting CLL cell survival and proliferation. Ibrutinib covalently binds C481 on BTK to inhibit BTK activity and hence inhibit survival and proliferation of CLL cells. B) Mutation of BTK C481S prevents covalent binding of ibrutinib to BTK and inhibits ibrutinib activity. The HDAC inhibitor abexinostat releases HDAC mediated suppression of miR-210 and miR-425 leading to a decrease in BTK expression and suppression of downstream signaling, even in the presence of C481S mutation.

Conflict of interest disclosure: The authors declare no competing financial interests

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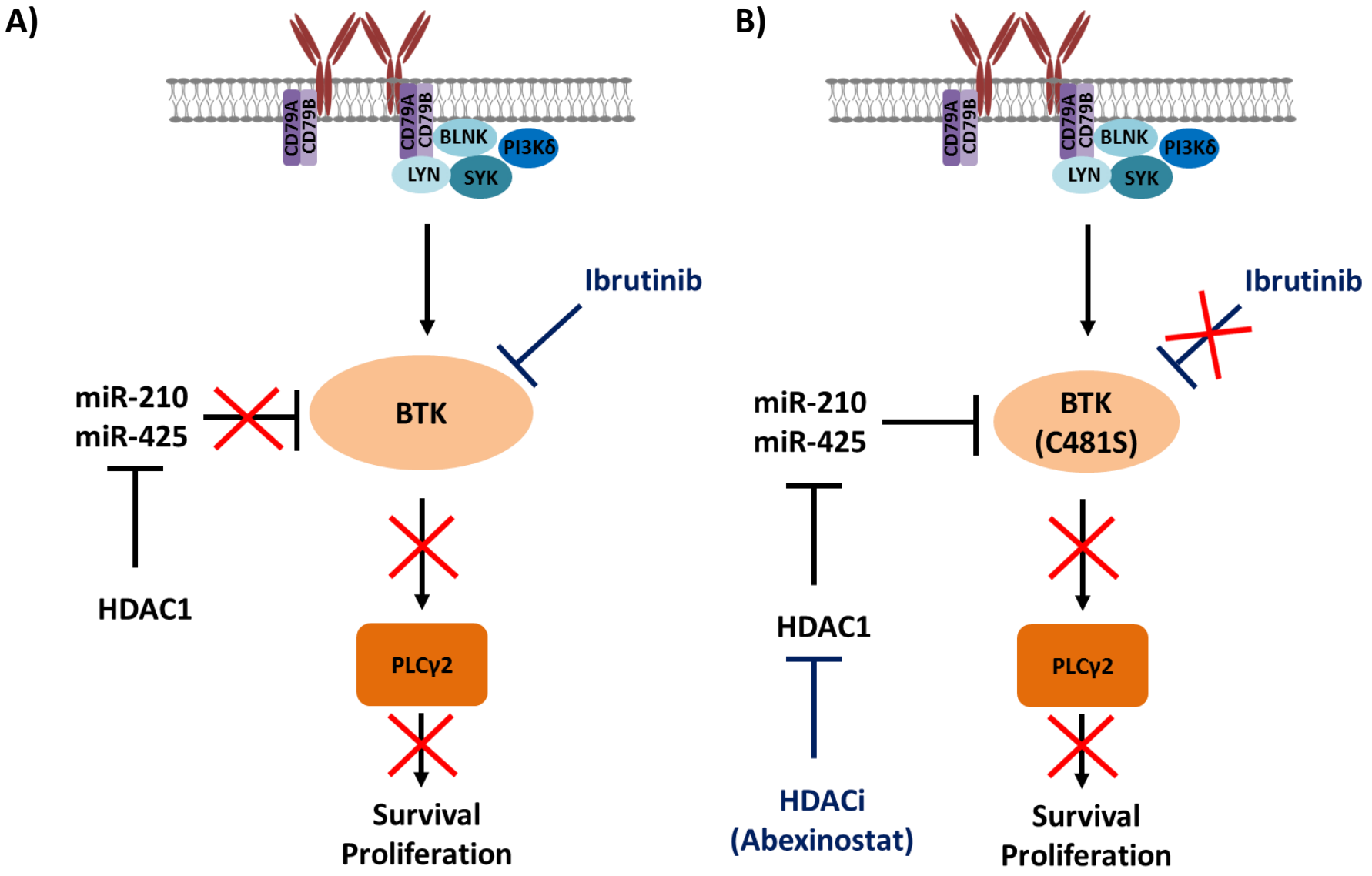
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